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(57) Abstract

Provided is a cotton *Gossypium hirsutum* promoter region from an expansin gene expressed in developing fiber.

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PLANT EXPANSIN PROMOTER SEQUENCES

INTRODUCTION

Technical Field

This invention relates to a cotton expansin promoter encoding sequence, and its use in contructs useful in modifying cotton fiber phenotypes.

This invention particularly relates to methods of using *in vitro* constructed DNA transcription or expression cassettes capable of directing fiber-tissue transcription of a DNA sequence of interest in cotton to produce fiber cells having an altered phenotype, and to methods of providing for or modifying various characteristics of cotton fiber as well as the modified cotton fibers produced by the method.

Background

In general, genetic engineering techniques have been directed to modifying the phenotype of individual prokaryotic and eukaryotic cells, especially in culture. Plant cells have proven more intransigent than other eukaryotic cells, due not only to a lack of suitable vector systems but also as a result of the different goals involved. For many applications, it is desirable to be able to control gene expression at a particular stage in the growth of a plant or in a particular plant part. For this purpose, regulatory sequences are required which afford the desired initiation of transcription in the appropriate cell types and/or at the appropriate time

in the plant's development without having serious detrimental effects on plant development and productivity.

It is therefore of interest to be able to isolate sequences which can be used to provide the desired regulation of transcription in a plant cell during the growing cycle of the host plant.

One aspect of this interest is the ability to change the phenotype of particular cell types, such as differentiated epidermal cells that originate in fiber tissue, *i.e.* cotton fiber cells, so as to provide for altered or improved aspects of the mature cell type. Cotton is a plant of great commercial significance. In addition to the use of cotton fiber in the production of textiles, other uses of cotton include food preparation with cotton seed oil and animal feed derived from cotton seed husks.

Despite the importance of cotton as a crop, the breeding and genetic engineering of cotton fiber phenotypes has taken place at a relatively slow rate because of the absence of reliable promoters for use in selectively effecting changes in the phenotype of the fiber. In order to effect the desired phenotypic changes, transcription initiation regions capable of initiating transcription in fiber cells during development are desired. Thus, an important goal of cotton bioengineering research is the acquisition of a reliable promoter which would permit expression of a protein selectively in cotton fiber to affect such qualities as fiber strength, length, color and dyability.

Relevant Literature

Cotton fiber-specific promoters are discussed in PCT publications WO 94/12014 and WO 95/08914, and John and Crow, Proc. Natl. Acad. Sci. USA, 89:5769-5773, 1992. cDNA clones that are preferentially expressed in cotton fiber have been isolated. One of the clones isolated corresponds to mRNA and protein that are highest during the late primary cell wall and early secondary cell wall synthesis stages. John and Crow, *supra*.

Agrobacterium-mediated cotton transformation is described in Umbeck, United States Patents Nos. 5,004,863 and 5,159,135 and cotton transformation by particle bombardment is reported in WO 92/15675, published September 17, 1992. Transformation of *Brassica* has been described by Radke *et al*. (Theor. Appl. Genet. (1988) 75;685-694; Plant Cell Reports (1992) 11:499-505.

SUMMARY OF THE INVENTION

The invention provides a cotton (*Gossypium hirsutum*) promoter region from an expansin gene expressed in developing fiber. Novel DNA promoter sequences are supplied, and methods for their use are described for directing transcription of a gene of interest in cotton fiber using the promoter region from an expansin gene which is expressed in cotton fibe.

In efforts to identify genes critical to fiber development, we have initiated a program sequencing randomly selected cDNA clones derived from a library prepared from mRNA

harvested from fibers at the stage in which secondary wall synthesis approaches its maximum rate (approximately 21 dpa).

We have characterized a cotton (*Gossypium hirsutum*) cDNA clone which is a homolog of the expansin gene. The sequences of this cDNA clone is homologous to that of other expansin encoding sequences The 5′ genomic promoter region from this gene has been sequenced for approximately 2200 base pairs.

Thus, the application provides sequences and methods of use relating to modification of phenotype in cotton fiber using a promoter of the cotton expansin gene.

DESCRIPTION OF THE DRAWINGS

Figure 1. Nucleic acid sequences to approximately 2200 bases of the promoter region 5′ to the encoding sequences to the cotton fiber expansin gene.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the subject invention, novel constructs and methods are described, which may be used provide for transcription of a nucleotide sequence of interest in cells of a plant host, preferentially in cotton fiber cells to produce cotton fiber having an altered phenotype.

Cotton fiber is a differentiated single epidermal cell of the outer integument of the ovule. It has four distinct growth phases; initiation, elongation (primary cell wall synthesis), secondary cell wall synthesis, and maturation. Initiation of fiber development appears to be triggered by hormones. The primary cell wall is laid down during the elongation phase, lasting up to 25 days postanthesis (DPA).

Synthesis of the secondary wall commences prior to the cessation of the elongation phase and continues to approximately 40 DPA, forming a wall of almost pure cellulose.

The constructs for use in such cells may include several forms, depending upon the intended use of the construct. Thus, the constructs include vectors, transcriptional cassettes, expression cassettes and plasmids. The transcriptional and translational initiation region (also sometimes referred to as a "promoter,") contains the transcriptional and translational functional elements and the initiation control region derived from or obtainable from the expansin gene (Figure 1).

In some embodiments, the promoter will be modified by the addition of sequences, such as enhancers, or deletions of nonessential and/or undesired sequences. By "obtainable" is intended a promoter having a DNA sequence sufficiently similar to that of a native promoter to provide for the desired specificity of transcription of a DNA sequence of interest. It includes natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences.

A transcriptional cassette for transcription of a nucleotide sequence of interest in cotton fiber will include in the direction of transcription, the cotton fiber transcriptional initiation region from expansin, a DNA sequence of interest, and a transcriptional termination region functional in the plant cell. When the cassette provides for the transcription and translation of a DNA sequence of

interest it is considered an expression cassette. One or more introns may be also be present.

Other sequences may also be present, including those encoding transit peptides and secretory leader sequences as desired.

Downstream from, and under the regulatory control of, the expansin transcriptional/translational initiation control region is preferably a nucleotide sequence of interest which provides for modification of the phenotype of fiber. The nucleotide sequence may be any open reading frame encoding a polypeptide of interest, for example, an enzyme, or a sequence complementary to a genomic sequence, where the genomic sequence may be an open reading frame, an intron, a noncoding leader sequence, or any other sequence where the complementary sequence inhibits transcription, messenger RNA processing, for example, splicing, or translation. The nucleotide sequences of this invention may be synthetic, naturally derived, or combinations thereof. Depending upon the nature of the DNA sequence of interest, it may be desirable to synthesize the sequence with plant preferred codons. The plant preferred codons may be determined from the codons of highest frequency in the proteins expressed in the largest amount in the particular plant species of interest. Phenotypic modification can be achieved by modulating production either of an endogenous transcription or translation product, for example as to the amount, relative distribution, or the like, or an exogenous transcription or translation product, for example to provide for a novel function or products in a transgenic host cell or tissue. Of particular interest are DNA sequences

encoding expression products associated with the development of plant fiber, including genes involved in metabolism of cytokinins, auxins, ethylene, abscissic acid, and the like. Methods and compositions for modulating cytokinin expression are described in United States Patent No. 5,177,307, which disclosure is hereby incorporated by reference. Alternatively, various genes, from sources including other eukaryotic or prokaryotic cells, including bacteria, such as those from *Agrobacterium tumefaciens* T-DNA auxin and cytokinin biosynthetic gene products, for example, and mammals, for example interferons, may be used.

The termination region which is employed in the expression cassette will be primarily one of convenience, since the termination regions appear to be relatively interchangeable. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, may be derived from another source. The termination region may be naturally occurring, or wholly or partially synthetic. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens,* such as the octopine synthase and nopaline synthase termination regions. In some embodiments, it may be desired to use the 3' termination region native to the cotton fiber transcription initiation region used in a particular construct.

As described herein, in some instances additional nucleotide sequences will be present in the constructs to provide for targeting of a particular gene product to specific cellular locations.

A variety of techniques are available and known to those skilled in the art for introduction of constructs into a plant cell host. These techniques include transfection with DNA employing *A. tumefaciens* or *A. rhizogenes* as the transfecting agent, protoplast fusion, injection, electroporation, particle acceleration, etc. For transformation with *Agrobacterium*, plasmids can be prepared in *E. coli* which contain DNA homologous with the Ti-plasmid, particularly T-DNA. The plasmid may or may not be capable of replication in *Agrobacterium*, that is, it may or may not have a broad spectrum prokaryotic replication system such as does, for example, pRK290, depending in part upon whether the transcription cassette is to be integrated into the Ti-plasmid or to be retained on an independent plasmid. The *Agrobacterium* host will contain a plasmid having the *vir* genes necessary for transfer of the T-DNA to the plant cell and may or may not have the complete T-DNA. At least the right border and frequently both the right and left borders of the T-DNA of the Ti- or Ri-plasmids will be joined as flanking regions to the transcription construct. The use of T-DNA for transformation of plant cells has received extensive study and is amply described in EPA Serial No. 120,516, Hoekema, In: The Binary Plant Vector System Offset-drukkerij Kanters B.V., Alblasserdam, 1985, Chapter V, Knauf, *et al.*, Genetic Analysis of Host Range Expression by *Agrobacterium*, In: Molecular Genetics of the Bacteria-Plant Interaction, Puhler, A. ed., Springer-Verlag, NY, 1983, p. 245, and An, *et al.*, *EMBO J.* (1985) 4:277-284.

For infection, particle acceleration and electroporation, a disarmed Ti-plasmid lacking particularly the tumor genes found in the T-DNA region) may be introduced into the plant cell. By means of a helper plasmid, the construct may be transferred to the *A. tumefaciens* and the resulting transfected organism used for transfecting a plant cell; explants may be cultivated with transformed *A. tumefaciens* or *A. rhizogenes* to allow for transfer of the transcription cassette to the plant cells. Alternatively, to enhance integration into the plant genome, terminal repeats of transposons may be used as borders in conjunction with a transposase. In this situation, expression of the transposase should be inducible, so that once the transcription construct is integrated into the genome, it should be relatively stably integrated. Transgenic plant cells are then placed in an appropriate selective medium for selection of transgenic cells which are then grown to callus, shoots grown and plantlets generated from the shoot by growing in rooting medium.

To confirm the presence of the transgenes in transgenic cells and plants, a Southern blot analysis can be performed using methods known to those skilled in the art. Expression products of the transgenes can be detected in any of a variety of ways, depending upon the nature of the product, and include immune assay, enzyme assay or visual inspection, for example to detect pigment formation in the appropriate plant part or cells. Once transgenic plants have been obtained, they may be grown to produce fiber having the desired phenotype. The fibers may be harvested, and/or the seed collected. The seed may serve as a source for growing additional plants having the

desired characteristics. The terms transgenic plants and transgenic cells include plants and cells derived from either transgenic plants or transgenic cells.

The various sequences provided herein may be used as molecular probes for the isolation of other sequences which may be useful in the present invention, for example, to obtain related transcriptional initiation regions from the same or different plant sources. Related transcriptional initiation regions obtainable from the sequences provided in this invention will show at least about 60% homology, and more preferred regions will demonstrate an even greater percentage of homology with the probes.

Of particular importance is the ability to obtain related transcription initiation control regions having the timing and tissue parameters described herein. Thus, by employing the techniques described in this application, and other techniques known in the art (such as Maniatis, *et al.*, *Molecular Cloning,- A Laboratory Manual* (Cold Spring Harbor, New York) 1982), other encoding regions or transcription initiation regions of expansin as described in this invention may be determined. The constructs can also be used in conjunction with plant regeneration systems to obtain plant cells and plants; thus, the constructs may be used to modify the phenotype of fiber cells, to provide cotton fibers which are colored as the result of genetic engineering to heretofor unavailable hues and/or intensities.

Various varieties and lines of cotton may find use in the described methods. Cultivated cotton species include *Gossypium hirsutum* and *G. babadense* (extra-long stable, or

Pima cotton), which evolved in the New World, and the Old World crops G. *herbaceum* and *G. arboreum.*

EXAMPLES

The following examples are offered by way of illustration and not by limitation.

Example 1

cDNA libraries

An unamplified cDNA library was used to prepare the Lambda Uni-Zap vector (Stratagene, LaJolla, CA) using cDNA derived from polyA+ mRNA prepared from fibers of *Gossypium hirsutum* Acala SJ-2 harvested at 21 DPA, the time at which secondary wall expansin is approaching a maximal rate (13). Approximately 250 plaques were randomly selected from the cDNA library, phages purified and plasmids excised from the phage vector and transformed.

The resulting clones/inserts were size screened on 0.8% agarose gels (DNA inserts below 600bp were excluded).

Example 2

Isolation and Sequencing of cDNA Clones

Plasmid DNA inserts were randomly sequenced using an Applied Biosystems (Foster City, CA) Model 373A DNA sequencer.

Example 3

Northern and Southern Analyses.

Cotton plants (*G. hirsutum* cv. Coker 130) were grown in the greenhouse and tissues harvested at the appropriate times

indicated and frozen in liquid N_2. Total cotton RNA and cotton genomic DNA was prepared and subjected to Northern and Southern analyses as described previously (14).

Example 4

Identification, Differential Expression and Genomic Analysis of Cotton Expansin Genes

During the course of screening and sequencing random cDNA clones from a cotton fiber specific cDNA library, it was discovered that one cDNA clone was very active during primary cell wall developement and had homology to the protein encoded by the expansin genes.

This clone was then utilized as a probe for Northern blot analysis to determine the differential expression in cotton tissues and developing cotton fiber. The expansin gene encodes a mRNA which is expressed at high levels in developing fiber, beginning at approximately day 1 through primary cell wall development at approximately day 20 post anthesis.

Example 5

Genomic DNA

cDNA for the expansin clone was used to probe for genomic clones. Full length genomic DNA was obtained from a library made using the lambda dash 2 vector from Stratagene™, which was used to construct a genomic DNA library from cotton variety Coker 130 (*Gossypium hirsutum* cv. coker 130), using DNA obtained from germinating seedlings.

The cotton genomic library was probed with a expansin probe and genomic phage candidates were identified and

purified. Figure 1 provides an approximately 2200 base pair sequence of the expansin promoter region which is immediately 5′ to the expansin encoding region. The start of the expansin enzyme encoding region is at the ATG at base number 2297, and the genomic clone begins at base number 122 of Figure 1.

Example 6

Cotton Transformation

Construct Preparation

Promoter constructs comprising the expansin promoter sequences linked to a gene of interest and other genetic elements of interest can be prepared in any of a number of ways known to the art, such as by ligation.

Explant Preparation

Coker 315 seeds are surface disinfected by placing in 50% Clorox (2.5% sodium hypochlorite solution) for 20 minutes and rinsing 3 times in sterile distilled water. Following surface sterilization, seeds are germinated in 25 x 150 sterile tubes containing 25 mls 1/2 x MS salts: 1/2 x B5 vitamins: 1.5% glucose: 0.3% gelrite. Seedlings are germinated in the dark at 28°C for 7 days. On the seventh day seedlings are placed in the light at 28±2°C.

Cocultivation and Plant Regeneration

Single colonies of *A. tumefaciens* strain 2760 containing binary plasmids pCGN2917 and pCGN2926 are transferred to 5 ml of MG/L broth and grown overnight at 30°C. Bacteria cultures

are diluted to 1 x 10^8 cells/ml with MG/L just prior to cocultivation. Hypocotyls are excised from eight day old seedlings, cut into 0.5-0.7 cm sections and placed onto tobacco feeder plates (Horsch *et al.* 1985). Feeder plates are prepared one day before use by plating 1.0 ml tobacco suspension culture onto a petri plate containing Callus Initiation Medium CIM without antibiotics (MS salts: B5 vitamins: 3 % glucose: 0.1 mg/L 2,4-D: 0.1 mg/L kinetin: 0.3% gelrite, pH adjusted to 5.8 prior to autoclaving). A sterile filter paper disc (Whatman #1) was placed on top of the feeder cells prior to use. After all sections are prepared, each section was dipped into an *A. tumefaciens* culture, blotted on sterile paper towels and returned to the tobacco feeder plates.

Following two days of cocultivation on the feeder plates, hypocotyl sections are placed on fresh Callus Initiation Medium containing 75 mg/L kanamycin and 500 mg/L carbenicillin. Tissue is incubated at 28±2°C, 30uE 16:8 light:dark period for 4 weeks. At four weeks the entire explant is transferred to fresh callus initiation medium containing antibiotics. After two weeks on the second pass, the callus is removed from the explants and split between Callus Initiation Medium and Regeneration Medium (MS salts: 40mM KNO_3: 10 mM NH_4Cl:B5 vitamins:3% glucose:0.3% gelrite:400 mg/L carb:75 mg/L kanamycin).

Embryogenic callus is identified 2-6 months following initiation and was subcultured onto fresh regeneration medium. Embryos are selected for germination, placed in static liquid Embryo Pulsing Medium (Stewart and Hsu medium: 0.01 mg/l NAA:

0.01 mg/L kinetin: 0.2 mg/L GA3) and incubated overnight at 30°C. The embryos are blotted on paper towels and placed into Magenta boxes containing 40 mls of Stewart and Hsu medium solidified with Gelrite. Germinating embryos are maintained at 28±2°C 50 uE $m^{-2}s^{-1}$ 16:8 photoperiod. Rooted plantlets are transferred to soil and established in the greenhouse.

Cotton growth conditions in growth chambers are as follows: 16 hour photoperiod, temperature of approximately 80-85°, light intensity of approximately 500μEinsteins. Cotton growth conditions in greenhouses are as follows: 14-16 hour photoperiod with light intensity of at least 400μEinsteins, day temperature 90-95°F, night temperature 70-75°F, relative humidity to approximately 80%.

Plant Analysis

Flowers from greenhouse grown T1 plants are tagged at anthesis in the greenhouse. Squares (cotton flower buds), flowers, bolls etc. are harvested from these plants at various stages of development and assayed for observable phenotype or tested for enzyme activity.

The above results demonstrate how the expansin cDNA may be used to alter the phenotype of a transgenic plant cell, and how the promoter may be used to modify transgenic cotton fiber cells.

All publications and patent applications cited in this specification are herein incorporated by reference as if each

individual publication or patent application are specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail, by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art that certain changes and modifications may be made thereto, without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. An isolated DNA sequence to a plant expansin promoter region of cotton.

2. The promoter encoding sequence of Claim 1 wherein said cotton expansin promoter region is taken the from sequence of Figure 1.

3. A recombinant DNA construct comprising any of the DNA encoding sequences of Claims 1-2.

4. A plant cell comprising a DNA construct of Claim 3.

5. A plant comprising a cell of Claim 4.

6. A method of modifying fiber phenotype in a cotton plant, said method comprising:

transforming a plant cell with DNA comprising a construct having the promoter of Claim 3 linked to a gene of interest,

wherein said gene of interest is capable of modifying a fiber characteristic when expressed in a fiber cell.

```
                   20                  40                  60                  80
         *         *         *         *         *         *         *         *
TACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGCTCGAGCGGCCGCCAGTGTGATGGATATCTGCAGAATTC

                  100                 120                 140                 160
         *         *         *         *         *         *         *         *
GGCTTACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGTATCCATATTCCATTTTTATATTATTTATTTTTAATATAA

                  180                 200                 220                 240
         *         *         *         *         *         *         *         *
TTATTATTTTTTTAATTATTTTAAAAATTTTAAATTTCCATTAACCTAATTTTTTTCTAAATAAATTATTCATATAAATA

                  260                 280                 300                 320
         *         *         *         *         *         *         *         *
AAGAAATTAAAAATACTAACATTTTAAAAATCTCAAAAATTCGTTATTCGAAAATTATTGTTTTCCTGAAATATATATAT

                  340                 360                 380                 400
         *         *         *         *         *         *         *         *
GAAAAATACTTTAAAATTTTATCAAATAATATTTAAAATATATAAAATAAAGTTCATTTTGTCAACATAAATTTAGAAAT

                  420                 440                 460                 480
         *         *         *         *         *         *         *         *
AAAAAACTAAAAATTAATATGAAAAAAATTGATAACCAAACTAAACTAAATTATAATTAAAAGTTCAAAAAAATTAAAAAA

                  500                 520                 540                 560
         *         *         *         *         *         *         *         *
ATCCCGATTGAACCAGTATCACCCCTAAATTAGATGAGGCCATATTTAACATATTAGAAAATGAAACTCTAGAAAAATAT

                  580                 600                 620                 640
         *         *         *         *         *         *         *         *
ATAAAAGTAAATTTATTGGCGAGAGATTAGACAAAGTCAATGCACCCCTCAATGAATAGATATTATTCCCAATGAAAGTT

                  660                 680                 700                 720
         *         *         *         *         *         *         *         *
TCCGTTTTCCACCTCCTACCCAAAAACTCCAAAGTCTCCAGAAGACGCGCCTGAATCGTGACTGGGTAGCCGGGGTACAT
```

FIG. 1A

```
             740                 760                 780                 800
   *          *         *         *         *         *         *         *
ACTACAACCCTAAATGCTTTATAGCGCATAGATCATGGGTTTAGCTTTGGATCCCATAAAGTACAAATACTGAGGTTCTT

             820                 840                 860                 880
   *          *         *         *         *         *         *         *
TAGTGAATGATCATGCATGGACACATGATGTCTCTTTTTAGGCATTTGACAAACTCGTCATTTTTCATACAATTTCTTT

             900                 920                 940                 960
   *          *         *         *         *         *         *         *
GGTCAATTAAATTTTCCATTAAATTAGGAAACCGCCCTCCAAAATTATANTGGTAACGGTGGAAGGNTTATCCAANTTCG

             980                1000                1020                1040
   *          *         *         *         *         *         *         *
ACCATTCGAACCACTTTTAAAAAATTAGAAAGTTATAATTTTTTTTCAAAAAAACTATAATACCTCTCTAGTTTTAGCT

            1060                1080                1100                1120
   *          *         *         *         *         *         *         *
AATTAAATTATTATTATTTTATTATTTTATTGTTATTAAAAGTGTAACTTGCACTCAACTATTAGTAAGTTTACGTTTTG

            1140                1160                1180                1200
   *          *         *         *         *         *         *         *
ATCACTTAATTTCAGAAAGTTAAAAAATGGTCTTTGAACTATTCGAAAATTTTCATTTAAGTTACTGGAATATTTAAAAG

            1220                1240                1260                1280
   *          *         *         *         *         *         *         *
TTTTTATTTAAGTCACCGGGCTATTAAGTTTTTTTTAAAAATTCGATTAGCAAGTTCCAAGCTACGATTCGATAAGTGA

            1300                1320                1340                1360
   *          *         *         *         *         *         *         *
TACAATGGATTTATACTTATTGACAAATAGAATATACATTAGGTCCAAGTTGATATTACGGTCAGTGTTGAAAATCGAAA

            1380                1400                1420                1440
   *          *         *         *         *         *         *         *
AAAAATATTTGGATTTTGATTCATAAATTTATGACTTCAAAGCTGGTTCATGAAAAAGAACTAAAGTGTAGGAGGGAAGG
```

FIG. 1B

```
                 1460                 1480                 1500                 1520
         *         *         *         *         *         *         *         *
AAAAAAATATCTTTTGATTGGCACAAACAGTGCGAACAAAGAAGACCACACAATAACAATTTTAACAATATACTAATTTA

                 1540                 1560                 1580                 1600
         *         *         *         *         *         *         *         *
AATGAAAAATTTTCAATAATTTAATAAGTTAACCGAGGAAAACTTACTAAGAGTTAGTTACCCCTGTTAAAATAACTTTC

                 1620                 1640                 1660                 1680
         *         *         *         *         *         *         *         *
ATGAAGTAATAGAAACTTTTAGTACGTATCATCTTATATAGAACAATTTCTATTTTCAGAAAGTCAAGAAAATTGTATTC

                 1700                 1720                 1740                 1760
         *         *         *         *         *         *         *         *
TAGAAAATGGCGACTTCTTCACCTTCAGTCCTTCCCTGATCGGCGCTTGTGAAAAACGAAAAACCTGAGTCTGATTGGCT

                 1780                 1800                 1820                 1840
         *         *         *         *         *         *         *         *
GACTGAAAATGAACCTACTCATCACCATTCACTATTACCAACTTCAAATGATAGGGGAATTAACTGGTAAAGTGTAACTC

                 1860                 1880                 1900                 1920
         *         *         *         *         *         *         *         *
CACCGATGGTTGAGGTGGTTGGCTGGAGTTAAATGAGATTTTTTTAGTTTTGTTTCAAGTGGCTTCAATTGCAAGCAATT

                 1940                 1960                 1980                 2000
         *         *         *         *         *         *         *         *
AGGAGACTGCGCTGGAATAACCCCTCGCTCAACCTTCCGCCATTGTTATGGTTTAATTAAACATTATGTTTCCATCCATC

                 2020                 2040                 2060                 2080
         *         *         *         *         *         *         *         *
TATATTTATATCCATTAAAACAAGTCGTTGAGCAAATAATGGATACTGGATACCATCATATCTATGATTAAAATTTTGCA
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FIG. 1C

```
                 2100                2120                2140                2160
        *         *         *         *         *         *         *         *
TGTGCCCTTTTAATGTATAGCTTAAGCCTTAATTATCCTCCAAATTTGTACTCTTTCACCACTTAATTAGCTACGTACGG

                 2180                2200                2220                2240
        *         *         *         *         *         *         *         *
TACTTAGCGTTGCTTGTCATCTTCTGTACTACAAACTCTTTCTCATTTTGTATAAATAGCTATACACTTTTTCTCTCCTC

                 2260                2280                2300                2320
        *         *         *         *         *         *         *         *
AAATCAATAAGGTTAGGTCAGCCAATTGTTTGAGCTAGCTAGCTCTTACTCAAATGGCAACCAAAACGATGATGTTGCAA

                 2340                2360                2380                2400
        *         *         *         *         *         *         *         *
ATATTTCCACTTTTCTTCTTTTTGTTCAGTGTCTGCAACTCCATTTTCCTTGGTGCTAATGGAGATGACAATGGTGGTTG

                 2420                2440                2460                2480
        *         *         *         *         *         *         *         *
GCAAACTGCCCATGCCACCTTCTACGGTGGTGCTGATGCTACCGGCACAATGGGTGAGTTTCAAACTTTCAAACCATTAC

                 2500                2520                2540                2560
        *         *         *         *         *         *         *         *
GGCATTACCTACATAAAAATCTCTAGGCTATGTTCTTAATTTGTGATGTTCTCTATAGGGGGAGCTTGTGGTTATGGAAA

                 2580                2600
        *         *         *         *         *
CCTGTACAGTCAAAGCCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGA
```

FIG. 1D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/00151

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N5/10 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 495 070 A (JOHN MALIYAKAL) 27 February 1996 see sequence ID no. 26 see column 63 - column 66 ---	1-6
A	SHIMIZU, YOSHINORI ET AL: "Changes in levels of mRNAs for cell wall-related enzymes in growing cotton fiber cells" PLANT CELL PHYSIOL., vol. 38, no. 3, 1997, pages 375-378, XP002064957 see page 377, column 2 see figure 3B ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 1998	29/05/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/00151

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOHN, MALIYAKAL E: "Gene expression in cotton (Gossypium hirsutum) fibers" ADV. PLANT BIOTECHNOL. BIOCHEM., 'PROC. INT. CONF. BIOTECHNOL. AGRIC. FOR.! (1993), 27-32. EDITOR(S): LODAH, M. L. PUBLISHER: IND. SOC. AGRIC. BIOCHEM., KANPUR, INDIA. CODEN: 60RTAG, XP000610459 see the whole document	1-6
A	WO 95 08914 A (AGRACETUS) 6 April 1995 see page 8, line 13 - page 23, line 25	1-6
A	WO 96 40924 A (CALGENE INC ;MCBRIDE KEVIN (US); STALKER DAVID M (US); PEAR JULIE) 19 December 1996 see the whole document	1-6
A	WO 96 40951 A (CALGENE INC) 19 December 1996 see the whole document	1-6
A	WO 96 35442 A (PENN STATE RES FOUND) 14 November 1996 see the whole document	1-6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte   ional Application No

PCT/US 98/00151

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			US	5620882 A	15-04-1997
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